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Lot Quality Assurance Sampling of Sputum Acid-Fast Bacillus Smears for Assessing Sputum Smear Microscopy Centers

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Assessment of 12 microscopy centers in a tuberculosis unit by blinded checking of eight sputum smears selected by using a lot quality assurance sampling (LQAS) method and by unblinded checking of all positive and five negative slides, among the slides examined in a month in a microscopy centre, revealed that the LQAS method can be implemented in the field to monitor the performance of acid-fast bacillus microscopy centers in national tuberculosis control programs.

Several measures are being undertaken by tuberculosis (TB) control programs worldwide to ensure the quality of sputum acid-fast bacillus (AFB) microscopy services that they provide (9). In India, in accordance with the Revised National Tuberculosis Control Programme (RNTCP) guidelines, all of the positive and five of the negative slides among the total slides examined in a calendar month in a designated microscopy center (DMC) are checked in an unblinded fashion by the senior TB laboratory supervisor (STLS) during his or her on-site supervisory visits (3). Recently, the international guidelines on external quality assessment of sputum AFB microscopy (7) have suggested a lot quality assurance sampling (LQAS) method, which is designed to recheck a minimum number of slides examined in DMCs to identify the faulty centers with an unsatisfactory level of performance. The present study documents the assessment of 12 microscopy centers in a subdistrict by using the two methodologies.

Organization. The organization of the RNTCP in India is shown in Fig. 1. Tiruvallur district in Tamil Nadu state has six subdistricts known as TB units (TUs). The Velliyur TU, where the present study was carried out, has 12 microscopy centers: seven DMCs and five non-designated microscopy centers (NDMCs). The collection of sputum samples, staining by a hot Ziehl-Neelsen method, and grading were done according to standard procedures (1). One trained laboratory technician is employed at each microscopy center, and one STLS is responsible for supervision of all microscopy centers, usually five in all, in the respective TUs. Direct sputum smears prepared during April 2002 and March 2003 were included in the study.

Unblinded checking. Every month, during on-site supervisory visits to the microscopy centers, the STLS in an unblinded fashion checks all positive smears and five negative smears (2, 3). The percentages of false-positive (any positive read as negative by the STLS) and false-negative (any negative read as positive by STLS) results were calculated. Centers with >5% error were considered to have systematic problems.

Blinded checking using LQAS. In July 2003, all of the routinely examined slides (from April 2002 to March 2003) were brought to the national reference laboratory at Tuberculosis Research Centre (TRC) Chennai for rechecking. From each microscopy center, 8 slides per month (annual total of 96 slides) were systematically selected after the slide number was recorded in the register. The sample size was based on the following considerations: a sensitivity of 80% (i.e., the percentage of detection of positive smears including low positives by the LT relative to the controller), a specificity of 100% (meaning that there should not be any false-positive error for LT), an acceptance number of zero, a slide positivity rate (SPR) of 10%, and a negative smear volume of 1,000 to 5,000 slides (7). The smears were checked in blinded fashion and the discrepant results were resolved by using an umpire reading. The umpire was provided with the smear results but the identity of the readers was concealed. The results of the umpire were final. The errors as defined in the recently published international guidelines are shown in the Table 1 and were followed (7). The SPR and the annual negative slide volume were calculated. The SPR for a center is the percentage of positives out of total slides examined in a year, and the annual negative slide volume is the total slides minus the positive slides. The absence of any error among the 96 slides in a microscopy center indicates that it had achieved the satisfactory level of performance. The occurrence of an error (Table 1), in particular a high false-positive (HFP) or high false-negative (HFN) result, indicates systemic problem(s) in the respective microscopy center.

Results and discussion. In unblinded checking of slides during on-site evaluation, all of the laboratories were judged to be doing well if the percentage of total error did not exceed 5%. Since bias in selection and checking of smears cannot be ruled out, unblinded checking does not, however, provide a true assessment of the performance of the respective microscopy center. Onsite supervision and evaluation of slides is primarily

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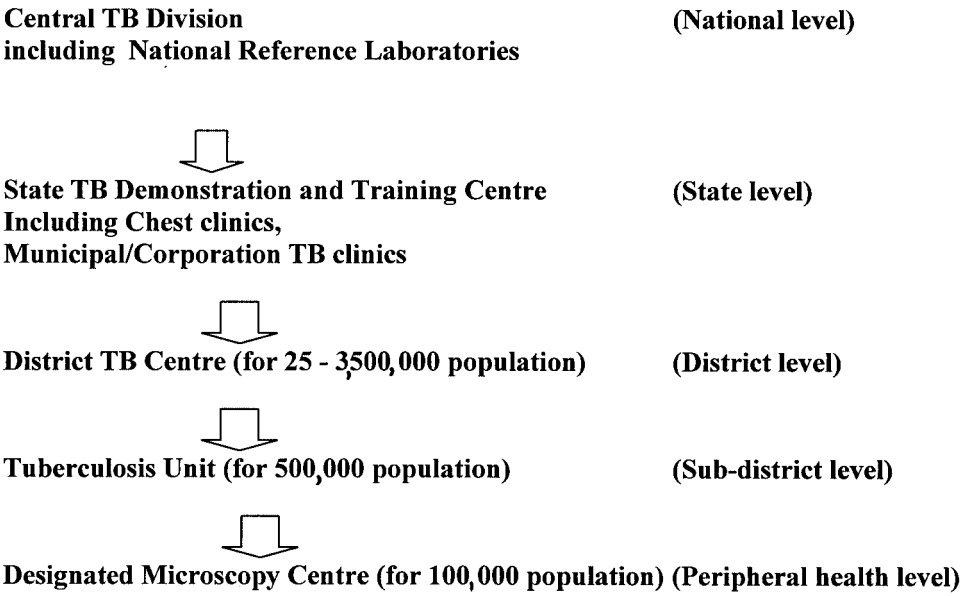

FIG. 1. Flow chart showing organization of RNTCP in India.

intended to motivate the laboratory technicians in microscopy centers and the results of unblinded checking cannot be considered to ensure the quality of performance.

The LQAS method, developed in industry to ensure the quality of products, is increasingly being applied in public health (4, 6). Its main advantage is the qualitative discrimination that it provides of the quality of sputum AFB work in microscopy centers, while requiring the rechecking of the minimum number of slides. For an average SPR (11%; range, 7.3 to 17.6%) and negative slide volume (1,480; range, 305 to 7,814), an annual sample size of 96 was to be checked (7). Accordingly, 2 centers—one (T) with one HFP and one HFN and another (X) with one HFN—were inferred to have a systemic problem, which required investigation and 5 (L, P, R, W, and Z) of 12 centers had no errors (Table 2). The numbers of errors for laboratory technicians at the TRC (HFP, 0; HFN, 2; LFP, 1; LFN, 4; and QE, 0) and for laboratory technicians in microscopy centers (HFP, 1; HFN, 2; LFP, 4; LFN, 1; and QE, 1) were seven and nine, respectively, thus ensuring the quality of checking.

It is also interesting that evaluation of the same centers during the same period adopting lot sampling of 20 slides per month per center also showed center T to have a systematic problem (10). Although the number and the method of selection of slides, the controllers, and the umpires were different, center T was identified as faulty by both the methods for rechecking of routine slides.

TABLE 1. Comparative description of errors[a]

Results obtained by technician	Result obtained by controller				
	Negative	Scanty	1+	2+	3+
Negative	C	LFN	HFN	HFN	HFN
Scanty	LFP	C	C	QE	QE
1+	HFP	C	C	C	QE
2+	HFP	QE	C	C	C
3+	HFP	QE	QE	C	C

[a] Negative, no AFB in 100 fields; Scanty, 1 to 9 AFB in 100 fields; 1+, 10 to 99 AFB in 100 fields; 2+, to 1 to 9 AFB per field in at least 50 fields; 3+, more than 10 AFB per field in at least 20 fields. Results: C, correct result; LFP, low false positive; LFN, low false-negative; QE, quantification error.

The performance of these 12 microscopy centers in the preceding 11 quarters (July 1999 to March 2002) was assessed by the STLS during on-site unblinded checking of all positive and 10% of negative slides (9). The number of slides per month checked by the STLS was very high (range of 180 to 413, including positive and negative slides). The performance of the same microscopy centers from April 2002 to March 2003 was also evaluated by checking 20 slides per center per month, which required the checking of 240 slides per month (10). The LQAS of 8 slides per month per center requires only 96 slides per month to be checked and thus would considerably reduce the workload for the STLSs. This reduction of workload could result in the improvement of quality of checking by the STLSs.

In RNTCP, each DMC is upgraded by allotment of a separate room for sputum AFB work and by providing a binocular microscope, quality reagents, and materials in addition to monitoring the quality of work by continuous supervision. On the other hand, the NDMCs are functioning with monocular microscopes and the quality of work is not supervised. In the present study, the NDMCs T and X performed poorly and were likely to offer poor quality of diagnostic services. These observations should be taken into account when microscopy services are organized in remote areas serving lower than the stipulated population as the number of suspects since the slides to be examined may be too few for the laboratory technician to maintain their proficiency.

The average sputum AFB smear positivity rate in the country is ca. 11%, and the negative slide volume in a majority of the microscopy centers is expected to range from 1,000 to 5,000 (5). Adopting the criteria explored in the present study, LQAS of eight slides per center per month would suffice to assess the

TABLE 2. Assessment of microscopy centers according to different checking methods

Criterion	DMCs							NDMCs				
Centre code	K	L	P	N	Q	R	S	T	X	W	Z	Y
Total no. of slides	8,715	828	1,395	2,742	1,301	1,491	644	1,058	332	359	341	740
Total no. of negative slides	7,814	682	1,279	2,304	1,206	1,339	579	971	286	314	305	686
SPR (%)	10.3	17.6	8.3	15.9	7.3	10.2	10.0	8.2	13.8	12.5	10.6	7.3
Type of error (no.) obtained by LQAS												
HFP								1				
HFN								1	1			
LFP	1			2			1					1
LFN												1
QE					1							
Total no.	1			2	1		1	2	1			2
Type of error obtained by onsite checking												
Total no. of positives checked	901	146	116	438	95	152	65	87	46	49	36	54
No. of false-positive results	4	1					2		1			1
% False-positive results	0.1	0.6	0	0	0	0	3	0	2.1	0		1.8
Total no. of negatives checked	60	60	60	60	60	60	60	60	60	60	60	60
No. of false-negative results	9	1					2	2	4	2	2	
% False-negative results	15	1.5	0	0	0	0	3.3	3.3	6.6	3.3	3.3	0
Total no. of errors	13	2					4	2	5	2	2	1
Total error (%)	1.3	1.0	0	0	0	0	3.2	1.4	4.7	2.0	2.0	0.9

performance of the majority of the microscopy centers in the country. Further field studies on implementing LQAS method into the external quality assessment process of the RNTCP in India are required urgently.

Conclusion. Implementation in the field of LQAS of eight slides per month per microscopy center appears to be operationally feasible under RNTCP and can be utilized for assessing the performance of the sputum smear microscopy centers.

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